

## EFFECT OF LIGHT ON LEAF INCLINATION OF *TRITICUM AESTIVUM*

### III. Seedling Age and Photosensitive Region

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As shown in the previous papers (Kimura 1974, 1975), blue light promotes blade inclination of the first leaf in wheat plant, and the maximum inclination occurs when the plants are exposed to 1600 erg/cm<sup>2</sup>/sec or more of blue light at 20–25°C for 48 hours. It was also shown that the inclination responses differ greatly depending on the age of seedlings at the start of blue light treatment. Maximum response was observed 9 days after germination when the plants were grown in darkness at 20°C.

In the present experiments, effect of blue light on blade inclination was examined in detail with etiolated seedlings at various ages. Furthermore, some experiments were conducted to find photosensitive site of leaves.

#### MATERIAL AND METHODS

The material used was the seedling of *Triticum aestivum*, "Shirasagi Komugi". Plants in plastic trays were grown on vermiculite at 20°C. The intensity of light used was about 2000 erg/cm<sup>2</sup>/sec at the plant level. Procedure of experimentation and light source were similar to those described in the previous paper (Kimura 1974).

#### RESULTS

##### *Seedling age*

##### 1) *Age in days*

In the present experiment, plants were grown in darkness at 20°C for 6–12 days and subsequently exposed to continuous blue light for 24 and 48 hours. Observations were made immediately after the light treatment.

Results shown in Fig. 1 indicate that the inclination responses to blue light differ greatly depending on the age of the seedling. In both 24- and 48-hour treatments, inclination response appeared in the 6-day-old seedlings and it increased to the maximum in 8- or 9-day-old seedlings, older seedlings being less sensitive. Changes in photosensitivity as influenced by the seedling age, were greater in the experimental group in which blue light was given for 48 hours than in the group in which blue light was given for 24 hours. No significant response was observed when laminar joint was still enclosed in the coleoptile.

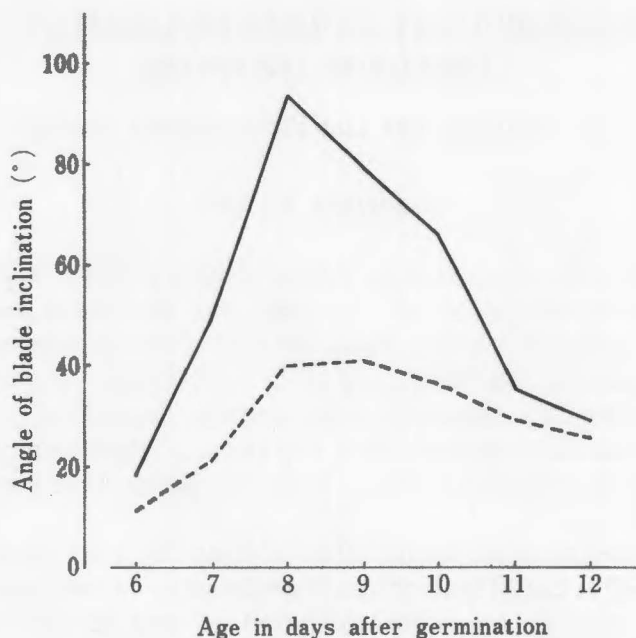


Fig. 1. Effect of seedling age on inclination response in the first leaf of wheat plants. Plants were cultured in darkness at 20°C for 6-12 days, and then exposed to blue light for 24 and 48 hours.

----- : 24-hour treatment, ——— : 48-hour treatment.

## 2) Effect of removal of coleoptile

In the above-mentioned experiment, young seedlings whose laminar joint of the first leaves were still in coleoptile failed to show the inclination response. In the present experiments, the effect of removal of coleoptile on the inclination response was investigated. Plants cultured in darkness at 20°C for 3-10 days were divided into two groups, each containing the plants at various ages. One group was removed the coleoptile just before blue light treatment, while the other one was not. To prevent the falling of the blade and leaf-sheath, the apical parts of the leaf blade were cut off by scissors, leaving 5 cm of the blade. After the plants were subjected to blue light for 48 hours, the inclination angles were measured. Results are shown in Fig. 2.

In the present experiment, the laminar joint of the intact plants appeared above the top of the coleoptile on the 7th day after germination. If the plants were deprived of coleoptile, the blade inclination was promoted by blue light to some extent, even in 3- to 6-day-old seedlings. Even in control plants cultured in darkness, the inclination was slightly promoted by removal of the coleoptile. The inclination response to blue light showed the maximum value 9 days after germination in both intact and treated plants.

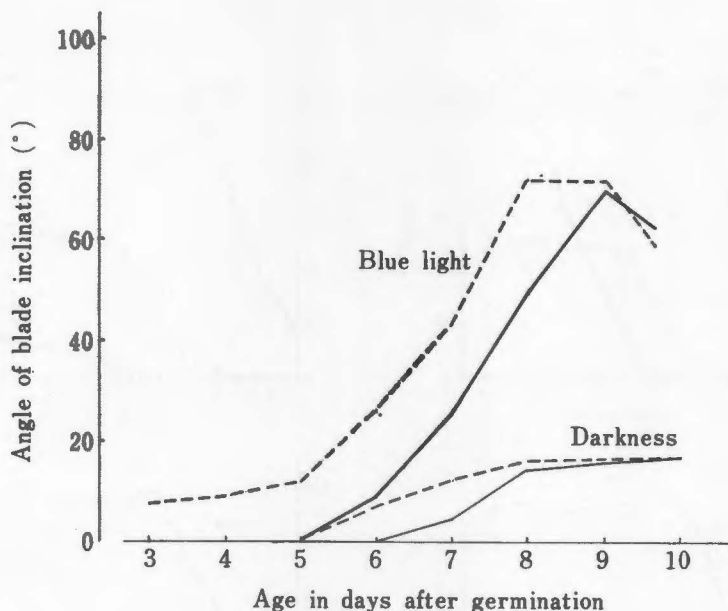


Fig. 2. Effect of removal of coleoptile on leaf-blade inclination of wheat plants irradiated by blue light. Plants were cultured in darkness at 20°C for 3-10 days, and then the coleoptile was removed. Immediately after cutting the coleoptile at various ages, plants were exposed to blue light for 48 hours.

-----: Plants deprived of coleoptile, —: Intact plants.

### 3) Seedling age and growth pattern

In the present experiment, relation between the seedling age which affects the photosensitivity and the growth pattern of the seedlings was examined. Plants were cultured under continuous irradiation of various colored lights and total darkness at 20°C. Intensity of the lights was adjusted to 2000 erg/cm<sup>2</sup>/sec at the plant level. Every day during the experimental period, 10 plants were sampled at random to examine the length of coleoptile, leaf sheath, leaf blade and blade angle of the first leaves. (Fig. 3).

Under any colored light, coleoptile elongation stopped 1 day after germination, and then leaf blade elongated rapidly and attained almost its maximum length at 4-5th days. After the leaf blade attained almost its maximum length, leaf sheath began to elongate and nearly reached their maxima at 7-8th day. These growth pattern was similar in all colored lights tested. However, in total darkness, the elongation of coleoptile stopped 2-3 days after germination, and its final length was somewhat larger than those in colored lights.

The leaf sheath elongated rapidly in darkness during the 4th and 8th day after germination, and its final length which was attained on 8th day

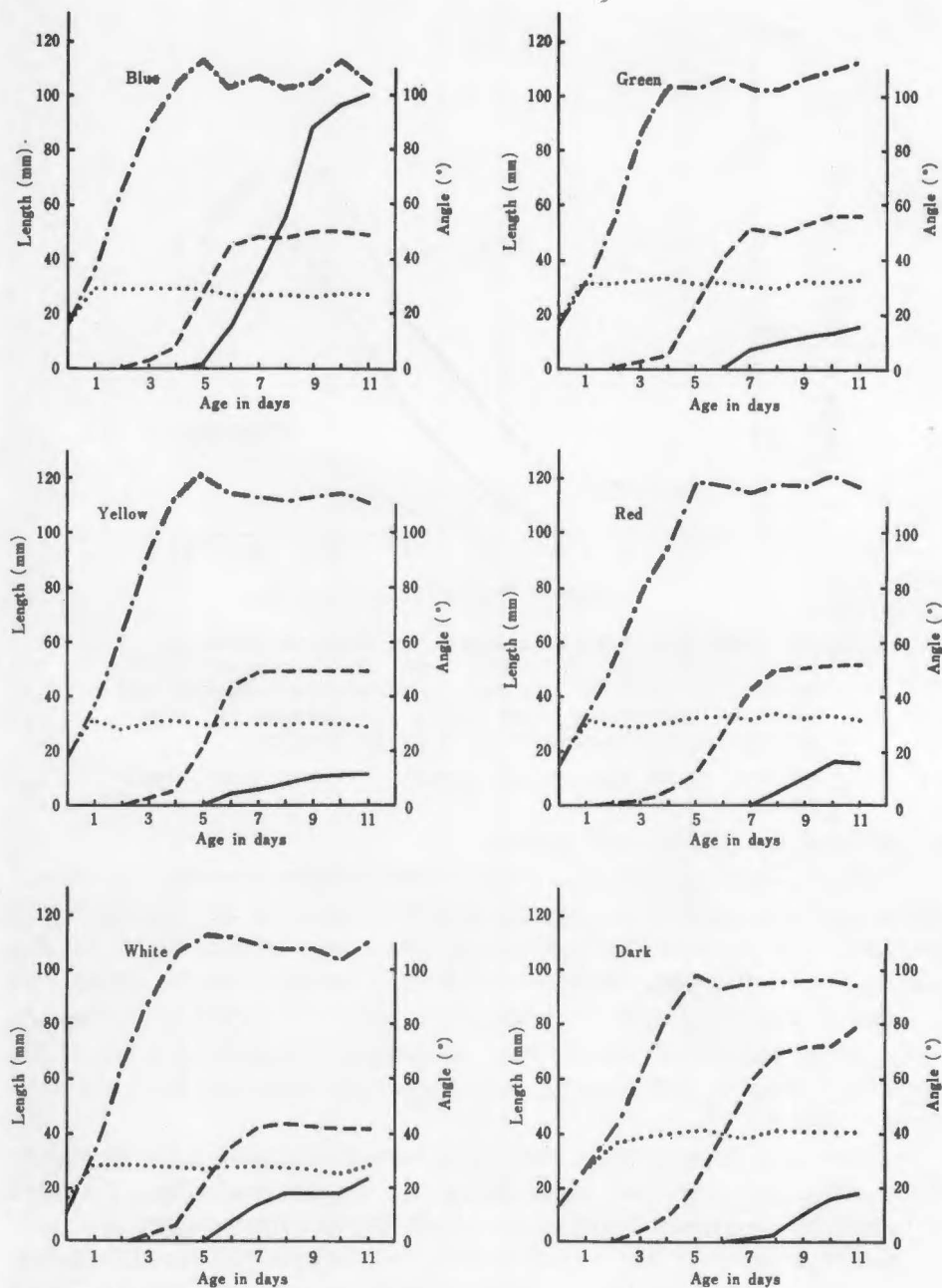


Fig. 3. Growth pattern and inclination responses of the first leaves in wheat plants cultured under various colored lights at 20°C.

—: Angle of blade inclination, .....: Length of coleoptile  
 - - - - : Length of leaf sheath, - · - · - : Length of leaf blade

was considerably larger than that in light. The laminar joint appeared from the top of coleoptile on the 6th day after germination in blue, green, yellow and white lights, and on the 7th day in red and darkness.

In all groups, leaf sheath was stopped the elongation 1-2 days after the appearance of the laminar joint. Leaf blade began to inclinate when laminar joint appeared from the top of coleoptile, and the maximum elongation of the blade occurred on 8-9th day when the leaf sheath stopped the elongation. The inclination angle increased greatly by blue light, but not much by green, yellow, red and white light, as had been reported in the previous paper (Kimura 1974).

#### *Photosensitive site*

The results of the experiments mentioned above suggest that the laminar joint is the photosensitive region in inclination response. The following experiments were designed to determine the photosensitive region of leaf in wheat plants which cultured in darkness for 8 days.

##### *1) Effect of removal of leaf blade*

To examine the role of the leaf blade in the inclination response, the apical parts of the first leaf blade were cut off by a scissors, leaving 0.5, 1 and 5 cm of the blade. Thereafter, the plants were subjected to blue light for 48 hours, and the inclination response was compared with that of intact plants. As the control, plants deprived of the apical parts of the blade were kept in total darkness. Results are shown in Fig. 4.

No significant difference in blade inclination were found between the intact and treated plants. This suggests that the leaf blade does not play an important role in the control of blade inclination.

##### *2) Shading of various regions of leaf*

To obtain more detailed information on photosensitive region of leaf, the following experiments were conducted. After leaf blade, laminar joint or leaf sheath were painted by black marker\* to shade from the light, plants were subjected to blue light for 48 hours. Control plants with black marker on the same regions of leaves were grown in darkness to examine the effect of black marker itself. The inclination responses are shown in Fig. 5.

When the shading treatment was given to the leaf blade and/or leaf sheath, leaving laminar joint, the blade inclination was nearly the same as that of the plants without shading. If the laminar joints were shaded, however, effect of the light was greatly diminished. As the blade inclination in darkness was not affected by black marker, it is considered that the black marker used in the present experiment has no effect on the blade inclination by itself.

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\*Vaseline paste containing carbon black.

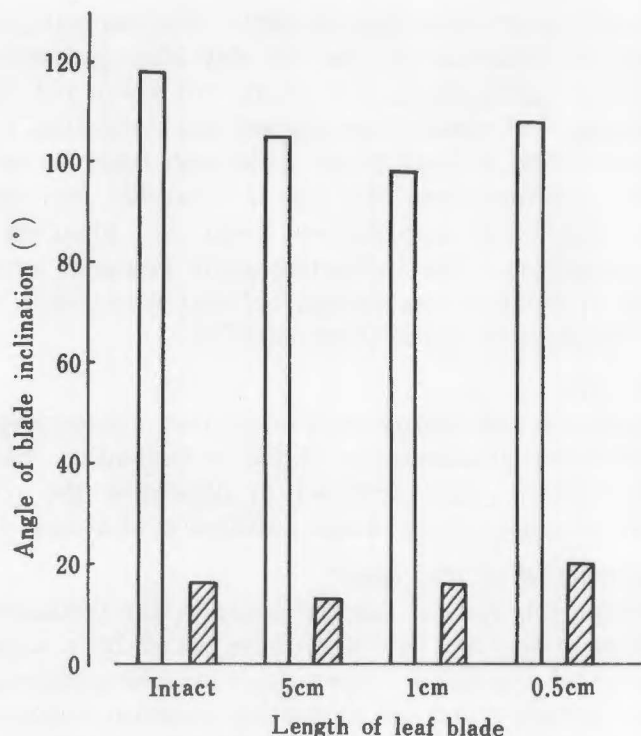


Fig. 4. A role of leaf blade on the leaf blade inclination of wheat plants irradiated with blue light. Plants were cultured in darkness for 8 days at 20°C, and then apical parts of the first leaf blade were cut off by a scissors, leaving 0.5, 1 and 5 cm. Immediately after the treatments, plants were exposed to blue light or kept in darkness for 48 hours.

□ Blue light irradiation,      ▨ Darkness

In another experiment, laminar joint was covered with floss-silk dyed black to shade from blue light irradiation. Result of this experiment was similar to that shown in Fig. 5, though the data is not presented in this paper.

From the results of these experiments, it is noticed that photosensitive region of leaf in inclination response is the laminar joint itself.

### 3) Irradiation with narrow beam.

Effect of localized irradiation was examined by giving narrow beams to different regions of the leaf. The seeds were sown in 18×70 mm glass tubes containing vermiculite. The tubes with one seed in each were immediately subjected to darkness at 20°C for 8 days, and then the apical parts of the 2nd leaf blades were removed by cutting 5 mm below the joint of the 1st leaves. Then, plants were exposed to blue beam of ca. 1000 erg/cm<sup>2</sup>/sec for 48 hours. In the preceding experiments, all

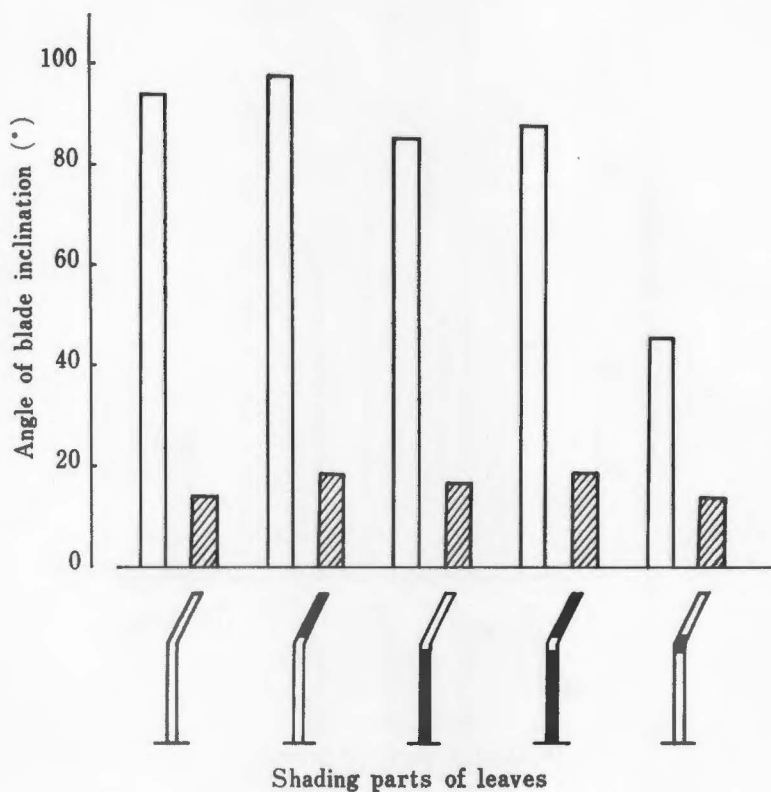


Fig. 5. Effect of shading given to various regions of leaves on leaf blade inclination of wheat plants irradiated with blue light. Plants were cultured in darkness at 20°C for 8 days, and then various parts of the first leaves were painted by black marker. Immediately after the treatments, plants were exposed to blue light or kept in darkness at 20°C for 48 hours.

□ Blue light irradiation,      ▨ Darkness

irradiations were given from the top. In the present experiments, however, horizontal narrow beam was given through a slit of ca. 1 mm width, prepared on one side wall of the box (30×30×20 cm). Each region of leaves to be irradiated was placed at 3 mm distance from the slit. The width of the irradiated region on the leaves was about 2 mm. Results are shown in Fig. 6.

Blue beam given to the laminar joint caused an increased inclination. The blue beam given to the leaf blade or sheath had some effect, but the effect was weak as compared with that of the beam given to the laminar joint.

Results also indicate the difference in sensitivity of the opposite side of the joint. Localized blue light given to the inner part of the joint caused deep inclination as compared with that given from the outer part.

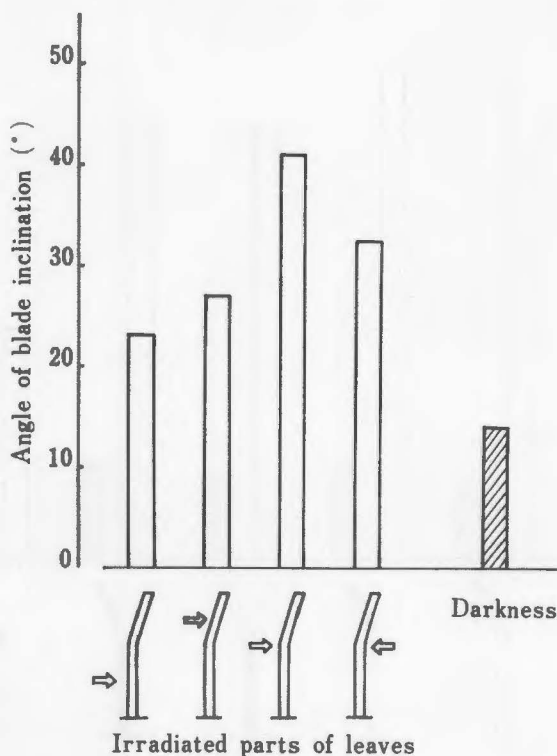


Fig. 6. Effect of blue light given to the limited parts of leaves on leaf blade inclination of wheat plants. Plants were cultured in darkness at 20°C for 8 days, and then various parts of leaves were irradiated with narrow blue beam (1000 erg/cm<sup>2</sup>/sec) for 48 hours.

⇒ : shows direction and region of irradiation.

The present experiments had some technical difficulty in giving the light beam to the definite region throughout the irradiation period. The leaf sheath elongated during the irradiation period, and the position of laminar joint changed, while the beam was fixed. Nevertheless, these results suggest that the site of photo-perception is laminar joint.

#### 4) Experiments with explants

Shading experiments were conducted with the excised first leaves. Plants were cultured in darkness at 20°C for 8 days, and the explants consisting of a laminar joint with 5 cm pieces of leaf blade and 5 cm pieces of leaf sheath were taken from the 1st leaves. Thereafter, each part of the laminar joint was painted by black marker, as shown schematically in Fig. 7, to examine the difference in sensitivity of each part to blue light. As the control, non-shaded explants were exposed to blue light and darkness. The explants were inserted vertically through the



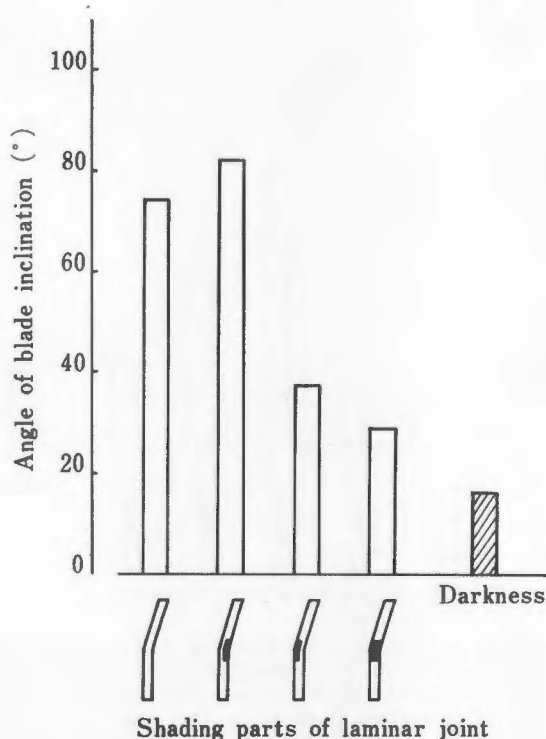


Fig. 7. Effect of shading given to various parts of laminar joint on blade inclination in excised wheat leaves irradiated with blue light. Explants were taken from the plants cultured in darkness at 20°C for 8 days. After various parts of laminar joint in explants were painted by black marker, they were exposed to blue light for 48 hours.

meshes of a plastic net covered on petri dishes containing dist. water. After a 48-hour treatment with blue light of 2000 erg/cm<sup>2</sup>/sec, the inclination responses were examined.

The blade inclination occurred in the explants, too, but showed somewhat smaller inclination than that in intact plants (cf. Fig. 5). Shading of the inner part only or both of the inner and outer parts of the joint inhibited the blade inclination. When only the outer part of the joint was shaded, inclination was nearly the same as that in non-shaded plants.

In the next experiment, effect of localized irradiation of the explants was examined by giving the blue beam of 1000 erg/cm<sup>2</sup>/sec for 72 hours. Procedure of experimentation and light sources were similar to those described above. To intercept the scattered light from other direction, whole part of joint in some cases and inner or outer parts of the joint in other cases were painted by blacked vaseline paste, and then different

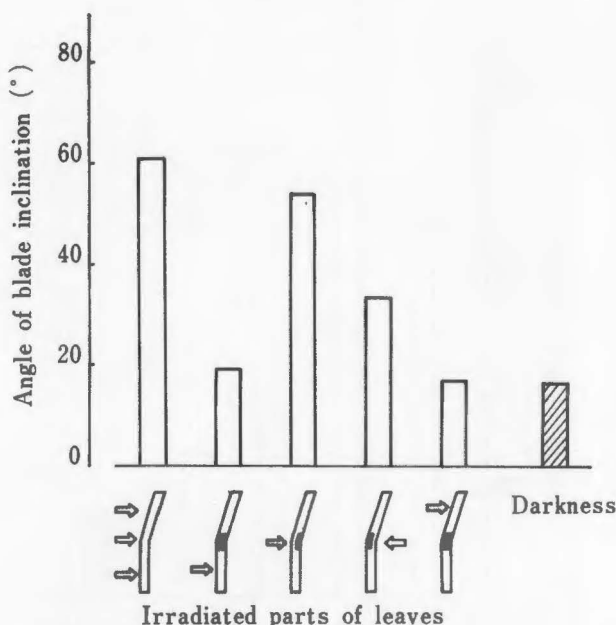


Fig. 8. Effect of blue light given to the limited parts of leaves on blade inclination of the excised wheat leaves. Explants were taken from the plants cultured in darkness at 20°C for 8 days. After various parts of laminar joint in the explants were painted by black marker, various parts of leaves were irradiated with narrow blue beam (1000 erg/cm<sup>2</sup>/sec) for 72 hours. ⇨: shows direction and region of irradiation.

region of the explants were irradiated with blue beam with 2 mm width. The treatments are shown schematically in Fig. 8.

The promotive effect of blue light on blade inclination was evident only when the laminar joint was exposed to light. In particular, blue light given to the inner part of the joint had strong effect. Irradiation given to the outer part of the joint was also effective to some extent, but the blue light given to the blade or the sheath had little effect.

From the results of these experiments, it may be concluded that the laminar joint itself is the photo-perceptive organ in inclination response, and its inner part is more sensitive than the outer one.

#### DISCUSSION

Inclination response (photonastic movement) of the first leaf blade in wheat was observed after 6-day growth in darkness, the maximum response being obtained on the 9th day. On the 6th day, laminar joint began to appear from the top of coleoptile, and on the 9th day the leaf sheath almost stopped the elongation. It is suggested that laminar joint itself is the site of photoperception in photonastic blade movement, and

that leaves whose leaf sheaths have just stopped elongation are most sensitive to light.

Results of the present experiments in which different regions of the leaves were shaded or narrow beam was applied to different regions, also indicated that the laminar joint itself is the photo-perceptive organ.

It is well known that the site of photoperception in phototropic response in oat coleoptile is the apex of coleoptile (Briggs 1963). In the case of lateral movement of leaves by asymmetric illumination in *Tropaeolum* and *Helianthus*, the petiole or the blade are most sensitive region for light perception (Brauner 1954). In tropic turgor movement or nyctinastic movements of some plants, the leaf blade is responsible for the perception of light (Yin 1937). Recently, Watanabe and Sibaoka (1973) reported that the pulvinule is the only site of photo-reception to opening response in *Mimosa* leaflets. Such was not the case in wheat plants.

Photonastic movement of wheat leaves presented here is irreversible, and differs from nyctinastic movements observed in many plants having pulvinous tissue. Similar photonastic movement of leaves was observed in rice plants (Inada 1969).

It is interesting that effect of blue light given to the inner part of joint was nearly the same as that given to whole part of leaf. This suggests that the inner part of joint is the photosensitive site. However, light given to the outer part was also somewhat effective. With regard to the effect of light given to the outer part of joint, two explanations are possible: 1) Outer part of joint is somewhat susceptible to light. 2) Light is transferred from the outer part of the joint to the sensitive inner part. Which alternatives is correct, is not determined from the results of the present experiment.

#### SUMMARY

1) In wheat seedlings grown in darkness at 20°C, the inclination response of the first leaf blade was observed after 6-day growth in darkness, and the maximum response was obtained on the 9th day.

2) Appearance of the photosensitivity was accompanied with the appearance of laminar joint above coleoptile tip, and the maximum sensitivity was attained when the elongation of the leaf sheath has just stopped.

3) Plants deprived of the apical parts of the blade were sensitive to light in the inclination response to the same extent as that of intact plants.

4) If the laminar joint was shaded by black marker, the inclination response was greatly inhibited, but the shading given to other regions of the leaf had no effect.

5) Localized irradiation of laminar joint with blue light caused strong blade inclination. The irradiation given to the inner part of laminar joint was more effective than that given to the outer part.

6) The blade inclination was observed even in the explants consisting of a laminar joint with 5 cm leaf sheath and 5 cm leaf blade, though the response was somewhat lower than that in intact plants.

7) From the results mentioned above, it was concluded that the photosensitive part of leaf in photonastic inclination response of wheat leaves is laminar joint itself, and its inner part is more sensitive than the outer one.

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